Remarks

SUMMARY OF THE RESPONSE

Applicants have amended claims 1, 3, 11, 13, 37 and 38 in order to direct the claims to expression of the preferred apoptosis inhibiting agents and preferred prodrug activating enzymes. Applicants have further amended these claims to make explicit that which was implicit, namely, that expression of the apoptosis inhibiting agent results in prolonging the lifespan of the neoplastic cell specifically for the purpose of increasing expression of a pro-drug activating enzyme. Support for these amendments can found throughout the specification, in particular in paragraphs [0042]-[0051] and [0130]-[0132].

Claim 1 has been amended to be directed to preferred apoptotic inhibitory agents which is based upon previous claim 6. Claim 3 has been amended to be directed to preferred prodrug activating enzymes, which is based upon previous claim 7. Accordingly, Applicants have cancelled claims 5, 6 and 7 without prejudice. Applicants reserve the right to continue prosecution of the cancelled subject matter in any continuation applications. Applicants have also amended claim 8 to preferred prodrug activating enzymes.

Accordingly, no new matter has been introduced by the amendments and their entry is respectfully requested.

DETAILED REMARKS

Claim Rejections - 35 USC § 102

The Examiner rejected claims 1, 3 and 5-6 under 35 U.S.C. 102(b) as allegedly being anticipated by Melcher *et al.* (British Journal of Cancer 78:144-145, 1998)("Melcher"). The Examiner alleges that Melcher teaches that further transfecting a colorectal tumor CMT93 cell line which is already transfected with HSV thymidine kinase (HSVtk), with a retrovirus encoding Bcl-2 to block apoptotic cell death during HSVtk/GCV killing. Specifically, the Examiner contends that although the Bcl-2 transfected cells remained sensitive to GCV *in vitro*, transfection of Bcl-2 greatly reduced amount of apoptotic cell death in the presence of ganciclovir ("GCV"). In particular, the Examiner contends on page 3 (lines 10-11) of the action

that Melcher indicates "coexpression of Bcl-2 with HSV thymidine kinase in tumor cells increases non-apoptotic cell death on ganciclovir treatment".

Applicants respectfully disagree, and submit that the rejection should be withdrawn for the reasons set forth below.

Melcher describes immunogenicity of tumor cells and demonstrates that by expression of Bcl-2 and inhibition of the apoptotic pathway, ganciclovir shifts its killing mechanism of colorectal cells from an apoptotic cell death mechanism to necrotic cell death, and leads to an increase tumor immunogenicity (see page 584, column 2 which states "the mechanism by which GCV kills HSVtk-expressing tumor cells can be diverted from apoptotic to predominantly non-apoptotic by manipulating the intracellular level of bcl-2"). No where in Melcher, is there any description or teaching that Bcl-2 prolongs the **life span** of the TK expressing colorectal cancer cell for a longer exposure to the drug ganciclovir. Rather, Melcher focuses on investigating a completely different phenomenon, namely, immunogenecity of tumor cells.

Furthermore, Melcher teaches that "the **proportion of apoptotic death was greatly reduced** at all time points" (page 582, 2nd col.) upon over-expression of *bcl*-2 and with GSV treatment. Thus, Melcher only teaches that the proportion of cell death attributed to necrotic and apoptotic cell death mechanism changes, but they reported no increase in total cell death. To the contrary, Applicants showed that the expression of an anti-apoptotic agent **increases total cell death** on treatment with a pro-drug.

Thus, based on what is taught in Melcher, a skilled artisan would not have used the method in the context Applicants claim its use. The phenomenon is now more precisely described in the claim and therefore, Applicants respectfully submit that the rejection is improper.

However, to expedite prosecution, Applicants have amended claims 1 and 3 to be directed to some preferred apoptotic inhibitor agent species, and preferred prodrug converting enzyme species which do not include bcl-2 or TK. Claims 5 and 6 have been cancelled without prejudice. Additionally, to further expedite prosecution, Applicants have amended claims 1 and 3, to be directed to the "apoptosis inhibiting agent results in **prolonged lifespan** of the neoplastic cell thereby increasing the expression of the prodrug activating enzyme" (emphasis added).

There is nothing in Melcher that teaches or suggests, or lets a reasonable person expect that a method as claimed would work in increasing the concentration of the prodrug converting enzyme.

Accordingly, Melcher does not teach all the elements of the present claims and the rejection of claims 1, 3 and 5-6 under 35 U.S.C. §102 over Melcher should be withdrawn.

Claim Rejections - 35 USC § 103

A. The Examiner rejected claims 1, 3, 5-11, 13 and 37-38 under 35 U.S.C. 103(a) as allegedly being unpatentable over Waxman et al. (WO 99/05299)("Waxman") in view of Bilbao et al. (WO 99/55382)("Bilbao"), Bullock et al. (Exp. Hematol. 21:1640-1647, 1993)("Bullock") and Melcher.

While the Examiner acknowledges that Waxman does not teach methods of killing neoplastic cells comprising the step of transducing neoplastic cells containing a vector encoding a prodrug activating enzyme with a vector encoding an apoptosis inhibiting agent, the Examiner contends that one of ordinary skill in the art would modify the teachings in Waxman to transduce a P450/RED expressing neoplastic cell with a vector encoding an apoptosis inhibiting agent such as bcl-2 based on the combined teaching of Bilbao, which allegedly teaches co-expression of bcl-2 gene to prolong transgene expression within the same cell, and the teachings of Bullock which allegedly teaches CPA expression reduces bcl-2 expression in human myeloid leukemia HL60 cells, with the teachings of Melcher, which allegedly teaches expression of bcl-2 to block apoptotic cell death during HSVtk/GCV killing.

Applicants respectfully disagree and submit that the rejection should be withdrawn for the following reasons.

As discussed previously, Melcher describes that **dual-transduced HSVtk/bcl-2 cells do not die through apoptosis** when exposed to GCV. This teaches directly away from the present invention. A skilled artisan reading Melcher is taught that HSVtk-expressing cells that also express bcl-2 continue to die (via necrosis) when exposed to a pro-drug, rather than the present invention, which is based upon the surprising discovery that the cells **survive longer** and are thus are better producers of the pro-drug conversion enzyme upon expression of an apoptosis inducing agent. Thus, without the knowledge gained from the present application, a skilled

artisan could not have envisioned that by adding an apoptosis inhibiting agent to a cell, one can broadly prolong the expression of a pro-drug converting enzyme.

Secondly, TK in combination with GSV differs from P450 in combination with CPA. In particular, the active metabolites formed by TK and GSV are phosphorylated nucleosides that remain trapped within the tumor cell as they are generated. Hence, unlike P450 and CPA, TK and GCV exhibits very little formation of soluble, diffusible metabolites. As a result, Melcher was able to effect a change in the tumor killing mechanism from apoptosis to necrosis upon overexpression of bcl-2. Importantly, the overexpression of bcl-2 in HSVtk cells did not result in an increased production of active, diffusible bystander cell-killing metabolites, but rather the GCV treatment of HSVtk cells expressing bcl-2 "still led to eradication of the tumor cell population within a **similar period of time**" (see Melcher, Fig 2b and page 582, col 2, line 3, emphasis added).

Additionally, in discussing Bullock, the Examiner is factually incorrect in alleging that **4-hydroperoxycyclophosphamide (4-HC)… is a P450-activated CPA** (p. 7, lines 1-2 of the 3/24/2009 Office Action). 4-HC is a chemically activated **derivative of CPA** which is also stated in Bullock "4-HC is an active derivative of cyclophosphamide" (see pg. 1640, first sentence of the introduction). 4-HC differs from P450-activated CPA in an important respect; **P450 activates CPA** to form 4-hydroxy-CPA (4OH-CPA). In contrast, 4-HC decomposes in aqueous medium to yield 4OH-CPA ***plus*** **hydrogen peroxide**. Additionally, 4-HC contributes substantially to apoptotic tumor cell death by stimulating the production of reactive oxygen species (ROS) which are **not** formed when CPA is activated by P450. Thus, the Examiner is incorrect in equating 4-HC with P450-activated CPA in terms of its effects on tumor cells, or the mechanisms of tumor cell death or its impact on the expression of factors such as Bcl-2 (see Appendix A; Murata et al., Free Radic Biol. Med. 2004; 37(6); 793-802). Accordingly, the studies reported by Bullock were carried out on a drug which is fundamentally different from P450-activated CPA.

Furthermore, 4-HC and P450-activated CPA are fundamentally different in terms of the doses required. In particular, the concentrations of 4-HC used in Bullock (i.e. 0.2 mM) are exceedingly high, supra-pharmacological concentrations that cannot be achieved **in vivo** or in cancer patients using CPA and are therefore not relevant. Moreover, Bullock discloses concentrations of 10, 20 or 50μM 4-HC **are without any effect** on DNA fragmentation and did

not induce apoptotic cell death (see Abstract). Rather, the use of 4-HC in Bullock are only relevant to the purpose of their study; ***ex vivo*** treatment of a patient's bone marrow cells with 4HC for the purpose of ***ex vivo*** purging of leukemic blasts from bone marrow followed by autotransplantation in leukemia patients. Accordingly, a skilled artisan would not have a reasonable expectation of success in light of the teachings of Bullock et al. due to the concentrations used in Bullock cannot be achieved ***in vivo***.

Accordingly, Applicants respectfully submit that one of ordinary skill in the art would not be able to modify the teachings in Waxman with the teachings in Bullock, Bilbao, and Melcher to come up with the present invention.

However, to expedite prosecution, Applicants have amended the claims as described, supra. As acknowledged by the Examiner, Waxman fails to teach methods of killing neoplastic cells comprising the step of transducing neoplastic cells containing a vector encoding a prodrug activating enzyme with a vector encoding an apoptosis inhibiting agent.

Bilbao only teaches co-expression of bcl-2 gene to prolong the transgene expression within the same cell, but fails to teach or discuss any method to increase expression of a prodrug converting enzyme or to teach or discuss use of any other apoptosis inhibiting agent to prolong survival of a neoplastic cell expressing a prodrug converting enzyme.

Bullock only teaches 4-HC treatment induces internucleosomal DNA fragmentation and is associated with a decrease in bcl-2 expression in human myeloid leukemia HL60 cells. In particular, Bullock states "HL60 cells that express relatively low levels of *bcl*-2, an exposure to 100μM/L 4-HC … produced a marked inhibition of *bcl*-2 expression and a concomitant decline in *c-myc* expression, thereby **facilitating induction of apoptosis**"(see page 1646, 1st col., lines 26-30; emphasis added). Accordingly, Bullock teaches **decreasing** the expression of *bcl*-2 facilitates 4HC-induced apoptosis, which is the direct opposite of the present invention whereby the expression of the apoptotic inhibiting agent in the neoplastic cells blocks drug-induced apoptosis of that cell. Bullock fails to teach or suggest any method to promote expression of *bcl*-2 (or any other anti-apoptotic gene) expression in 4-HC treated cells or to promote the survival of human leukemia HL60 cells by increasing the expression of *bcl*-2 or by any other an apoptosis inhibiting agent.

Melcher describes that expression of *bcl-2* blocks apoptotic cell death during HSVtk/GCV killing and increases immunogenicity of the HSVtk tumor cells. Thus, Melcher teaches directly away from the present invention. If anything, a skilled artisan reading Melcher would consider that there is no effect on cell survival when bcl-2 is added to a cell and the cell subjected to a pro-drug. Accordingly, also Melcher fails to teach or suggest use of bcl-2 to prolong the lifespan of the cell.

Accordingly, Applicants respectfully submit that the rejection of claims 1, 3, 5-11, 13 and 37-38 should be withdrawn.

B. The Examiner also rejected claims 14-18 and 31-33 under 35 U.S.C. 103(a) as allegedly being unpatentable over Waxman in view of Bilbao, Bullock and Melcher and further in view of Robertson *et al* (US 6,709,866)("Robertson") and Griffith *et al.* (US 6,900,185)("Griffith").

Applicants respectfully disagree and submit that the rejection should be withdrawn for the following reasons.

As described above, the combination of Waxman with Bullock, Bilbao and Melcher does not disclose all the elements of the present claims because they do not describe a method to promote the survival of a neoplastic cell to increase the expression of the prodrug converting enzyme.

Robertson and Griffith do not overcome this deficiency.

Robertson only teaches methods of neuroprotection and prolonging the lifespan of neurons by expressing NIAP and IAP polypeptides. Robertson fails to teach or suggest expression of NIAP and IAP polypeptides to prolong the survival of neoplastic cells, nor the use of expression of NIAP and IAP polypeptides to facilitate cell death by increasing the expression of prodrug converting enzymes

Griffith only teaches methods of **inhibiting tumor cell growth** by administering a vector comprising a DNA expression sequence encoding TRAIL, where expression of TRAIL results in **tumor inhibition**, and "rapidly leads to cell death by apoptosis" (see col 5, lines 8-9). Griffith fails to teach or suggest the expression of TRAIL in conjunction with the expression of a prodrug converting enzyme. Accordingly, Griffin teaches the opposite of the present invention. Griffin

teaches using a vector to express TRAIL to increase apoptosis of a tumor cell. Importantly, Griffin fails to teach or suggest the expression of TRAIL to promote the lifespan of a tumor cell or TRAIL or an inhibitor of TRAIL to **decreases apoptosis** in a tumor cell. Griffin also fails to disclose or discuss or teach the use of TRAIL to increase the production of a second transgene, such as a prodrug converting enzyme.

Therefore, because the combination of all the references does not teach all the elements of the current claims, the rejection of claims 14-18 and 31-33 under 35 U.S.C. 103(a) is improper and should be withdrawn.

Moreover, even assuming, *arguendo*, that the references taught all the elements of the claims, which they do not, there would not have been motivation to combine them. The Examiner contends that Robertson taught the use of a recombinant viral vector expressing various anti-apoptotic polypeptides such as NAIP, HIAP, HIAP2, XIAP and other under the control of a regulatable promoter to inhibit death of a cell of the nervous system in a patient. Applicants point out that the anti-apoptotic polypetides in Roberston were used for a completely different purpose, namely the anti-apoptotic polypeptides (i.e. NAIP, HIAP, HIAP2, XIAP etc) were expressed specifically to **promote survival** of a **neuronal cell.** As neurons are irreplaceable, one strategy to promote neuronal survival (i.e. to **prevent neuronal loss**), as taught in Robertson, is by overexpressing anti-apoptotic agents. In contrast to the present invention, under no circumstances would it be obvious to use anti-apoptotic polypeptides to **prevent the loss of tumor cells,** which is precisely the cell type one is trying to eliminate. Instead, one of ordinary skill in the art would likely use ***pro-***apoptotic polypeptides to promote the loss or killing of a tumor cell. Thus, the present invention directed to enhancing the killing of a tumor cell by **promoting survival** of the tumor cell is counter-intuitive to what an ordinary person of ordinary skill would do to enhance killing of a tumor cell.

Additionally, the Examiner contends that Griffith teaches a method of inducing tumor cell apoptosis using Trail/Apo2-L gene transfer in a mammal.

Accordingly, in view of the amendments to claims 11 and 13 to ensure the expression of the apoptosis inhibitory agent increases the lifespan of the neoplastic cell, Applicants respectfully submit that the rejection has been obviated.

C. The Examiner further rejected claims 1, 3, and 5-6 (with respect to the elected species p35) under 35 U.S.C. 103(a) as allegedly being unpatentable over Waxman in view of Bilbao, Bullock and Melcher, and further in view of Beidler et al. (J. Biol. Chem. 270:16526-16528, 1995)("Beidler").

The Examiner acknowledges that the teachings of Waxman, Bilbao, Bullock and Melcher do not specifically teach the use of a vector comprising a nucleic acid encoding an apoptosis inhibiting agent which is p35, but contends that these teachings could be modified in view of Beidler by using a vector comprising a nucleic acid encoding an apoptosis inhibiting agent which is baculovirus p35.

Applicants respectfully disagree and submit that the rejection should be withdrawn for the following reasons.

As discussed above, the combination of Waxman, Bilbao, Bullock and Melcher does not disclose all the elements of the present claims because they do not describe a method to promote the survival of a neoplastic cell to increase the expression of the prodrug converting enzyme.

Beidler does not overcome this deficiency. Beidler only teaches methods of p35 expression in MCF7 breast carcinoma cells to inhibit Fas and TNF-induced apoptosis. Beilder fails to suggest or teach expression of p35 in a cell expressing a prodrug converting enzyme, and furthermore, fails to teach or suggest expression of p35 to prolong the lifespan of a tumor cell in order to increase the expression of a prodrug converting enzyme or expression of p35 to facilitate in the long term the killing of the tumor cells.

Accordingly, in view of the above, the rejection under 35 U.S.C. §103(a) over Waxman in view of Bilbao, Bullock and Melcher, and further in view of Beidler, should be withdrawn.

In view of the foregoing amendments, arguments and evidence, Applicants respectfully submit that all claims are in condition for allowance. Early and favorable action is respectfully requested.

In the event that any additional fees are required, the Commissioner is authorized to charge Nixon Peabody LLP Deposit Account No. 50-0850.

Date: July 23, 2009

Respectfully submitted,

/Leena H. Karttunen/

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